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Dephytinisation of soyabean protein isolate with low native phytic acid content has limited impact on mineral and trace element absorption in healthy infants

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Infant formulas based on soyabean protein isolate are often used as an alternative to cows'-based formulas. However, the presence of phytic acid in soya formulas has raised concern about the absorption of trace elements and minerals from these products. The aim of the present study was to evaluate mineral and trace element absorption from regular and dephytinised soya formula in healthy infants. Soyabean protein isolate with a relatively low native content of phytic acid was used for production of a regular soya formula (300 mg phytic acid/kg liquid formula) and an experimental formula was based on dephytinised soya protein isolate (<6 mg phytic acid/kg liquid formula). Using a crossover study design, apparent mineral and trace element absorptions were measured by a stable isotope technique based on 72 h faecal excretion of non-absorbed stable isotopes (Zn, Fe, Cu and Ca) and by the chemical balance technique (Mn, Zn, Cu and Ca) in nine infants (69–191 d old). Fe absorption was also measured by erythrocyte incorporation 14 d after intake. The results from the present study demonstrated that Zn absorption, measured by a stable isotope technique, was significantly greater after dephytinisation (mean value 16.7 v. 22.6%; $P=0.03$). No other statistically significant differences between the two formulas were observed. The nutritional benefit of dephytinisation was marginal in the present study. Based on these results, the use of soyabean protein isolate with low native content of phytic acid should be promoted for production of soya formulas and adequate addition of ascorbic acid to enhance Fe absorption should be ensured in the products.

Soyabean: Phytic acid: Infant formula: Stable isotopes: Dephytinisation: Infants

Infant formulas based on soyabean protein isolate are frequently used as alternatives to cows' formulas, in particular in the USA where a large proportion of formula-milk-fed infants are fed soyabean-based products (for review, see Mendez *et al.* (2002)). Healthy western infants fed on soya formula grow and develop as well as their peers fed on cows' formula (for reviews, see Fomon (1993a) and Mendez *et al.* (2002)). However, earlier reports demonstrated that children recovering from severe malnutrition gained weight less well when fed on soya formula as compared with a diet based on cows' (Golden & Golden, 1981). These results were largely attributed to low Zn bioavailability from the soya formula due to the presence of phytate (myo-inositol hexaphosphate), a major storage form of P and a potent inhibitor of trace element absorption. Zn absorption was later demonstrated to be significantly lower from soya formula, as compared with human and cows' formulas in adults (Sandström *et al.* 1983). In addition, sodium phytate added to cows' formula

was shown to reduce Zn absorption in adults (Lönnerdal *et al.* 1984). Significantly lower Fe absorption from infant formulas based on soya, as compared with cows' formula, was also demonstrated in adult women (Derman *et al.* 1987).

During the last few years, the possibility of removing or degrading phytic acid from soyabean protein isolate, used as the protein source in infant formula, has been explored. However, the nutritional impact of dephytinised soya formula on mineral and trace element absorption has not been evaluated systematically in infants. Dephytinisation of soyabean protein isolate was demonstrated to be a useful approach to improve Fe bioavailability from soya formula in infants (Davidsson *et al.* 1994), but neither Zn nor Ca absorption were improved by partial reduction of phytic acid (Ziegler *et al.* 1990). However, an enhancing effect of dephytinisation of soyabean protein isolate on Mn absorption has been demonstrated in infants (Ziegler *et al.* 1990) and adults (Davidsson *et al.* 1995).

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The aim of the present study was to evaluate the effect of dephytinisation of soyabean protein isolate on the absorption of Fe, Zn, Cu, Ca and Mn in healthy infants fed soya formula. Soyabean protein isolate with a relatively low native content of phytic acid, before and after enzymatic degradation of phytic acid, was used for production of infant formulas. Phytic acid contents in the two products were 300 and <6 mg phytic acid/kg liquid formula respectively. Apparent absorption of Zn, Cu and Ca were measured by a stable isotope technique based on 72 h faecal excretion of non-absorbed stable isotopes after intake of 300 g labelled formula. Apparent absorption of Mn, Zn, Cu and Ca was evaluated by the chemical balance technique, based on dietary intake and faecal excretion of minerals and trace elements during 72 h. Fe absorption was measured by a stable isotope technique based on erythrocyte incorporation 14 d after intake, as well as by faecal monitoring. The study had a crossover design, i.e. each infant acted as his/her own control.

Subjects and methods

Subjects

The study protocol was approved by the University of Iowa Institutional Review Board and one or both parents gave written consent. Nine apparently healthy full-term infants participated in the study. Five of the nine subjects were girls and four were boys. The study infants included one set of twins and one set of triplets. On the first day of the first metabolic balance study, they ranged in age from 69 to 191 (median 93) d and had a mean body weight of 5.9 (range 4.4–7.4) kg. The infants had been fed formula since birth as the parents' decision. During the study they were fed the assigned study formulas exclusively, with the exception of one infant who, because of his age, was also fed limited amounts of commercially prepared infant foods. No cereal products were included in his diet.

Sample size calculations were based on our previous data on erythrocyte incorporation of Fe in infants (Davidsson *et al.* 1994). It was estimated that eight to ten infants would be a sufficient sample size to detect a nutritionally significant difference in erythrocyte incorporation of 50 % with 90 % power (ten infants) or 80 % power (eight infants) and a type I error rate of 5 %.

Study design

The study had a balanced crossover design in which each subject was studied once while receiving the experimental formula (dephytinised formula) and once while receiving the regular soya formula. The diets were fed in predetermined random order. Each study consisted of a 10 d washout period followed by a 3 d period during which a metabolic balance was conducted.

Formulas

The formulas were produced especially for the present study at the Nestlé Product Technology Centre (Konolfingen, Switzerland). The composition of the products was similar

to a commercial infant formula (Alsoy[®]; Carnation[®], Glendale, CA, USA), except for the use of dephytinised soyabean protein isolate in production of the experimental (dephytinised) formula. Regular and dephytinised formulas were each prepared in two batches. One batch, fed during the washout periods and during the metabolic balance studies, was fortified with Fe, Zn and Cu. The other batch, used for the preparation of labelled test meals, was prepared without added Fe, Zn and Cu. Dephytinised soyabean protein isolate was produced with the use of phytase from *Aspergillus niger* (Gist-Brocades, Delft, The Netherlands) from commercial soyabean protein isolate (Protein Technologies International, St Louis, MO, USA). The study formulas were prepared in ready-to-feed form.

Experimental procedures

After the 10 d washout period, infants were admitted to the Lora N. Thomas Metabolism Ward (Department of Pediatrics, University of Iowa, Iowa City, IA, USA) in the morning. After measurements of weight and length, infants were placed on specially designed metabolic beds. No less than 3 h after the previous feeding, they were fed 150 g isotopically labelled formula. This feed also contained 50 mg carmine. The remainder of the labelled formula (150 g) was fed as the next feed of the day (about 3 h later). No additional formula was fed until at least 3 h after intake of the second labelled test meal. Solid foods (one infant) were withheld for 6 h. The exact amount of formula consumed was determined by weighing feeding bottles before and after feeding. For the remainder of the 72 h metabolic balance period infants were fed unlabelled study formula. Formula fed exactly 72 h after intake of the first carmine-containing formula feed also contained carmine. Passage of carmine-stained stool following this second dose marked the end of the metabolic balance period. Infants stayed in the ward during the entire 72 h collection period. Faeces were collected into acid-washed Pyrex containers using methods described previously (Fomon, 1993b). Faecal samples were weighed, frozen and shipped on dry ice to Switzerland for analysis.

Blood samples and analysis of iron status indices

Samples of capillary blood (approximately 1 ml) were obtained before the first isotope administration in each study period and again 14 d later (total of four blood samples). Blood was obtained by heel prick using a disposable spring-loaded device (Tenderfoot; International Technidyne Corporation, Edison, NJ, USA) and collected in EDTA-treated tubes. Hb was determined using a Coulter Counter (model M430; Coulter Electronics, Hialeah, FL, USA). Plasma was separated from blood cells within 30 min of collection and stored at –20°C. Ferritin was analysed by RIA using the Quantimmune kit (catalogue no.190–2001; Bio-Rad Laboratories, Hercules, CA, USA).

Preparation of labelled test meals

Labelled test meals were prepared from infant formula produced without added Fe, Zn and Cu. Approximately 12 h

before a scheduled isotope administration, 1.2 mg ^{58}Fe , 0.25 mg ^{70}Zn , 0.25 mg ^{65}Cu and 5.0 mg ^{42}Ca were added to 300 g liquid formula. Test meals were stirred and kept refrigerated until fed.

Stable isotope labels

CaCO_3 (77.746 % ^{42}Ca), ZnO (70.55 % ^{70}Zn) and Cu metal (99.20 % ^{65}Cu) were purchased from Medgenix (Ratingen, Germany). Fe metal (91.727 % ^{58}Fe) was supplied by Isotec (St-Quentin, France). ^{70}ZnO , ^{65}Cu metal and ^{58}Fe metal were dissolved in stoichiometric amounts of 0.5 M- H_2SO_4 to prepare metal sulfates. $^{42}\text{CaCO}_3$ was dissolved in a stoichiometric amount of 0.5 M-HCl to obtain CaCl_2 . Total Ca, Fe, Zn and Cu were measured by flame atomic absorption spectrometry (model 975; Varian, Mulgrave, Victoria, Australia) using external calibration and instrument conditions recommended by the manufacturer. Ca was analysed after addition of lanthanum oxide (Fluka, Buchs, Switzerland) to a final concentration of 10 g La/l. Total concentrations were 2.607 mg Ca, 0.850 mg Fe, 154.9 μg Cu and 109.7 μg Zn/g solution. The isotopic composition of the labels was measured by thermal ionisation MS (Finnigan MAT Model THQ; Thermoquad, Bremen, Germany). The solutions were placed into acid-washed 5 ml Reacti vials (Pierce, Rockford, IL, USA) and kept refrigerated.

Sample preparation and analysis of faecal samples

Faecal samples were freeze dried, pooled, homogenised (Compact Robot; Tefal, Selongey, France) and stored in a desiccator until analysis. Pooled samples included the first faecal sample dyed by carmine and all consecutive faecal material up until, but not including, faecal material dyed by the second dose of carmine. Duplicate samples (approximately 300 mg) were dry ashed for 48 h in silica Erlenmeyer flasks in a muffle furnace at 520°C for measurements of total Ca, Cu, Zn, Mn and enrichments of ^{42}Ca , ^{65}Cu and ^{70}Zn . After cooling, 2 ml concentrated HNO_3 was added, acid was evaporated to dryness on a hot-plate and samples were heated in the muffle furnace overnight at 520°C. Ash was redissolved in 4 ml concentrated HCl and diluted to 25 ml. Ca and Cu contents were determined by flame atomic absorption spectrometry. Zn and Mn were measured by inductively coupled plasma MS using external calibration. A portion of the faecal digest was diluted to 10–100 μg Mn/l and 100–1000 μg Zn/l in 0.5 M- HNO_3 . Rh (20 μg /l) was used as internal standard. The inductively coupled plasma MS instrument (Perkin-Elmer Elan 6000; Perkin-Elmer Europe, Rotkreuz, Switzerland) was equipped with a GemTip™ cross-flow nebuliser and a Scott-type double-pass spray chamber and optimised by using a multielement solution (10 μg /l; Plasma Setup Solution, Perkin-Elmer). The operating conditions were as follows: radio frequency power 1000 W, nebuliser Ar flow rate 0.83 l/min, mass resolution 0.8 Da at 10 % peak height. The following data collection variables for Mn and Zn were used: detector mode, pulse counting; mode, peak hopping; selected isotopes, ^{64}Zn and ^{55}Mn ; dwell time, ^{64}Zn 10 ms, ^{55}Mn 20 ms; sweeps 100; number of replicates 5; sample uptake rate 1.2 ml/min.

Accuracy of analysis was verified by analysing the National Institute of Standards and Technology's (Gaithersburg, MD, USA) standard reference material bovine liver (1577b) and a pooled human faecal sample. CV for Cu, Zn and Mn were 3.4, 6.6 and 4.0 % (n 14; bovine liver) respectively and results were within the range of the certified values. CV for Ca, Cu, Zn and Mn were 5.2, 3.3 and 2.6 and 3.5 % respectively (n 10; in-house-prepared pool of faecal material). Zn was separated from matrix elements by anion exchange (resin AG1-X8, 200–400 mesh, Cl-form; Bio-Rad Laboratories, Glattbrugg, Switzerland) and Ca was precipitated with a saturated basic ammonium oxalate solution (Davidsson *et al.* 1996). Ca and Zn isotope ratios were determined by thermal ionisation MS. Instrumental mass discrimination was corrected for by measuring Ca and Zn isotope ratios of CaNO_3 and ZnNO_3 standards with natural isotopic composition (Merck, Darmstadt, Germany). The ratio correction factor was recalibrated for every batch of samples. Within-run precision was <0.5 % for $^{40}\text{Ca}/^{42}\text{Ca}$ and <1 % for $^{68}\text{Zn}/^{70}\text{Zn}$. Relative external precision determined by measuring baseline samples was 0.61 % for $^{40}\text{Ca}/^{42}\text{Ca}$ and 0.40 % for $^{68}\text{Zn}/^{70}\text{Zn}$ (n 17). After correction for instrumental mass discrimination, isotope ratios for basal faecal samples were within 0.8 % of the accepted International Union of Pure and Applied Chemistry values (Rosman & Taylor, 1998). Cu isotope ratios were determined by inductively coupled MS. Samples of digested faecal samples were diluted to 0.1 mg Cu/l with 0.5 M- HNO_3 . The following variables for data collection were used: detector mode, pulse counting and peak hopping; dwell time ^{63}Cu and ^{65}Cu 50 ms; sweeps 100; number of replicates five. Instrumental mass discrimination was corrected for by measurements of Cu isotope ratios in 0.1 mg Cu/l with natural isotopic composition (Cu standard solution; Merck). The ratio correction factor was recalibrated every ten samples. After correction for instrumental mass discrimination, $^{65}\text{Cu}/^{63}\text{Cu}$ isotope ratios for baseline faecal samples were within 0.30 % of the accepted International Union of Pure and Applied Chemistry value (Rosman & Taylor, 1998). Accuracy was also verified by spiking baseline faecal samples with known amounts of highly enriched ^{65}Cu . Regression analysis of data based on measured isotopic ratios and calculated isotopic ratios resulted in the following equation: $y = -0.00036 + 1.00153x$ (R^2 0.9997). The 95 % CI for the slope and intercept were (0.999324, 1.009821) and (−0.0664, 0.00593) respectively. Average within-run precision based on five replicates was 0.66 %. Repeatability determined by measuring baseline samples was <0.63 % (n 34). The detection limit for enrichment of ^{65}Cu in faeces calculated using the average internal relative standard deviation according to Patterson *et al.* (1999) was 0.89 %.

Total Fe and ^{58}Fe in faeces were determined by double-isotope dilution, as this technique was more precise than atomic absorption spectroscopy for analysis of Fe in faecal material (see later). Faecal samples were spiked with a known amount of enriched ^{57}Fe followed by measurements of the isotopic ratios $^{57}\text{Fe}/^{56}\text{Fe}$ and $^{58}\text{Fe}/^{56}\text{Fe}$. The ^{57}Fe -spike was prepared by dissolving 84.2 mg Fe metal (95.15 % ^{57}Fe) in 1 ml concentrated

HNO₃ + 4 ml H₂O. The solution was diluted to approximately 200 mg Fe/kg. Fe concentration of the solution was 203.26 (SD 0.50) mg/kg (*n* 4) as determined by reverse isotope dilution using a 200 mg Fe/l standard (Fe(NO₃)₂; Merck). Faecal samples (approximately 250 mg) were mineralised in Teflon perfluoralkoxy copolymer (PFA) digestion bombs (maximum pressure 1400 kPa) after addition of concentrated HNO₃ and H₂O₂ (300 ml/l) using a microwave digestion system with pressure control (MDS-2000; CEM Corp., Matthews, NC, USA). Solutions were diluted to 25 ml and a portion (7 g) was spiked with 0.3–1.0 g ⁵⁷Fe to obtain a ⁵⁷Fe/⁵⁶Fe isotope ratio of approximately 0.5–1.0 in the sample. Fe concentrations determined by atomic absorption spectroscopy during quantitative analysis of Ca and Cu were used for these calculations. Fe in spiked samples was separated from matrix elements by anion exchange chromatography followed by determination of isotope ratios by thermal ionisation MS as described previously (Kastenmayer *et al.* 1994; Davidsson *et al.* 2000). Within-run precision was <0.5 % for ⁵⁷Fe/⁵⁶Fe and <1 % for ⁵⁸Fe/⁵⁶Fe. Relative external precision was 0.7 % for ⁵⁷Fe/⁵⁶Fe and <1.3 % for ⁵⁸Fe/⁵⁶Fe (Merck; *n* 14). A pooled human faecal sample was analysed as an in-house laboratory standard. Total Fe determined by isotope dilution was 1343 (SD 12) mg/kg (CV 0.9 %, *n* 14) and agreed well with the value established by flame absorption spectroscopy 1311 (SD 90) mg/kg (CV 6.9 %, *n* 10). CV for ⁵⁸Fe and total Fe in the faecal pool were 1.8 and 0.9 % respectively. Blanks were measured by processing known amounts of ⁵⁷Fe-spike together with the samples to monitor sample contamination.

Sample preparation and analysis of blood samples

Samples of whole blood were analysed in duplicate for Fe isotopic composition according to Davidsson *et al.* (2000). Samples of whole blood (approximately 400 mg) were mineralised in a microwave digestion system (see earlier). Fe isotope ratios were determined by thermal ionisation MS after separation by anion exchange chromatography. Relative external precisions for ⁵⁴Fe/⁵⁶Fe, ⁵⁷Fe/⁵⁶Fe and ⁵⁸Fe/⁵⁶Fe ratios in baseline blood samples were 0.74, 0.39 and 0.51 % (*n* 8). Relative accuracies of the ⁵⁴Fe/⁵⁶Fe, ⁵⁷Fe/⁵⁶Fe and ⁵⁸Fe/⁵⁶Fe ratios of baseline blood samples after correction for fractionation were within 2 % of the accepted absolute Fe isotopic composition determined by the Central Bureau for Nuclear Measurements (Geel, Belgium; Taylor *et al.* 1992).

All acids were purified by sub-boiling in a quartz still (Kürner Analysentechnik, Rosenheim, Germany). Other chemicals were analytical grade purity. Only ultrapure water (18 MΩ) was used. To minimise contamination through vessel material, only acid-washed quartz, polytetrafluoroethylene and polyethylene containers were used.

Calculation of apparent absorption; stable isotopes and chemical balances

Fractional apparent absorptions of ⁴²Ca, ⁶⁵Cu and ⁷⁰Zn were calculated based on 72 h faecal excretion of stable

isotopes ingested with the labelled test meals (300 g formula), according to Turnlund *et al.* (1982). For ⁵⁸Fe fractional absorption, calculations were based on simplified equations derived from triple-spiking calculations described by Turnlund *et al.* (1993). The amount of ⁵⁸Fe incorporated into erythrocytes 14 d after administration of the labelled test meals was calculated as described in detail previously (Kastenmayer *et al.* 1994). Apparent absorptions of Ca, Cu, Zn and Mn were calculated based on the analysis of the total amount of elements in diet and faeces collected during the 72 h balance period.

Food analyses

Ca and Zn were analysed by flame atomic absorption spectroscopy after dry-ashing (see earlier). Cu and Mn contents were determined by graphite furnace atomic absorption spectrometry (SpectrAA 400 Zeeman; Varian) using standard addition to minimise matrix effects and microwave digestion. Pyrolytically coated graphite tubes (Varian) were used. The furnace programme included charring at 700°C (Mn), 800°C (Cu) and atomisation at 2400°C (Mn), 2300°C (Cu). The National Institute of Standards and Technology's standard reference materials wheat flour 1667a and non-fat powder 1549 were analysed for quality control. Phytic acid content (inositol phosphates 5 and 6) was determined by ion pair reverse-phase HPLC after extraction with 2.4 % HCl (Sandberg & Ahderinne, 1986; Sandberg *et al.* 1989).

Statistical analyses

Statistical differences were evaluated by ANOVA, with soya formula, period and sequence as fixed factors and subjects as the tested factor (Senn, 1993). The effects of study period and sequence were not significant (*P* > 0.05). Results are therefore presented as mean values with *P* values for differences between formulas and 95 % CI for the formula effect. For Fe absorption data, the CI are asymmetrical as the analysis was performed on log-transformed data in order to account for non-normality. Results for Fe erythrocyte incorporation and apparent Fe absorption based on faecal excretion were compared using paired *t* test. Data were analysed with NCSS 2000 (Number Cruncher Statistical System, Kaysville, UT, USA).

Results

All infant formulas contained 125–126 g solids, 2720 kJ (650 kcal), 32 g fat, 18 g protein and 150 mg ascorbic acid/kg liquid formula. Concentrations of phytic acid, minerals and trace elements are presented in Table 1. After addition of stable isotope labels, the total content of minerals and trace elements per 300 g formula was 179.3–180.6 mg Ca, 2.3–2.5 mg Fe, 2.5 mg Zn and 0.34–0.35 mg Cu. Molar ratios of phytic acid relative to the total mineral and trace element contents in the labelled regular soya formula were: Ca 0.03, Fe 3.05, Zn 3.57, Cu 25.5.

Mean Hb concentration was 118 (SD 13) g/l with three infants having concentrations <110 g/l. Geometric mean

Table 1. Concentrations of phytic acid, calcium, iron, copper, zinc and manganese in ready-to-feed soya formula

Formula*	Phytic acid (mg/kg)	Ca (mg/kg)	Fe (μ g/kg)	Cu (μ g/kg)	Zn (μ g/kg)	Mn (μ g/kg)
Regular (batch A)	250	600	7840	839	8440	305
Regular (batch B)†	300	581	2940	313	886	303
Dephytinised (batch C)	< 6	593	8620	738	8480	303
Dephytinised (batch D)†	< 6	585	2800	299	872	303

* Produced especially for the present study at the Nestlé Product Technology Centre (Konolfingen, Switzerland).

† Stable isotope labels were added before administration.

plasma ferritin was 35 μ g/l (+1SD 72 μ g/l, -1SD 17 μ g/l); no infant had plasma ferritin concentration < 12 μ g/l.

Results for mineral and trace element apparent absorptions are given in Tables 2 and 3. Zn absorption, measured by a stable isotope technique, was significantly greater from the dephytinised formula (mean value 22.6 %) than from the regular soya formula (mean value 16.7 %; $P=0.03$). However, apparent absorptions of Zn from dephytinised and regular soya formula based on 72 h chemical balance studies were not significantly different. No statistically significant differences in apparent absorptions of Cu or Ca were observed with either technique. Mean apparent absorption of Mn increased from 25.4 to 42.1 % (regular formula v. dephytinised formula); however, this difference was not statistically significant ($P=0.06$). Erythrocyte incorporation of Fe stable isotope did not increase significantly after dephytinisation (Table 4; mean values 6.3 v. 8.3 %,

$P=0.10$), nor did apparent Fe absorption measured by faecal monitoring. Apparent absorption of Fe, based on faecal excretion of the isotope, was significantly greater ($P=0.001$) than erythrocyte incorporation of Fe from both formulas (Table 4).

Discussion

The results from the present study demonstrate for the first time the inhibitory effect of phytic acid on Zn absorption in infants. These results are thus in agreement with our earlier observation in adults, as we found a significant increase in Zn absorption from soya formula after 100 % dephytinisation; mean Zn absorption increased from 12 to 22 % (L Davidsson, A Almgren, B Sandström, MA Juillerat and RF Hurrell, unpublished results). In addition, increased Zn absorption has been demonstrated in adults

Table 2. Apparent absorptions (%) of zinc, copper and calcium from regular and dephytinised soya formula in nine healthy infants, based on the stable isotope technique*

(Mean values and 95 % confidence intervals)

	Regular soya formula	Dephytinised soya formula	Statistical significance of effect: P	95 % CI
Zn	16.7	22.6	0.03	0.9, 10.8
Cu	31.2	35.0	0.34	-4.9, 12.6
Ca	64.2	65.6	0.57	-4.3, 7.2

* For details of formulas, subjects and procedures, see Table 1 and p. 288.

Table 3. Apparent absorptions (%) of zinc, copper, calcium and manganese from regular and dephytinised soya formula in nine healthy infants, based on the chemical balance technique*

(Mean values and 95 % confidence intervals)

	Regular soya formula	Dephytinised soya formula	Statistical significance of effect: P	95 % CI
Zn	21.1	28.4	0.20	-4.7, 19.2
Cu	41.7	38.7	0.49	-12.8, 6.7
Ca	48.6	55.9	0.12	-2.3, 16.8
Mn	25.4	42.1	0.06	-0.8, 34.2

* For details of formulas, subjects and procedures, see Table 1 and p. 288.

Table 4. Iron absorption (%), based on erythrocyte incorporation of stable isotopes 14 d after intake and on faecal excretion of non-absorbed isotopes during 72 h balances in nine healthy infants*

(Mean values and 95 % confidence intervals)

Methodology	Regular soya formula	Dephytinised soya formula	Statistical significance of effect: P	95 % CI
Erythrocyte incorporation	6.3	8.3	0.10	-0.4, 5.2
Faecal excretion	13.9	16.8	0.40	-4.2, 15.5

* For details of formulas, subjects and procedures, see Table 1 and p. 288.

after degradation of phytic acid in cereals. Nävert *et al.* (1985) reported significantly higher Zn absorption from bread containing wheat bran after prolonged leavening and Kivistö *et al.* (1989) reported significantly higher Zn absorption from extruded crispbread prepared from wheat bran with reduced phytic acid content. Recently, Zn absorption by adults from a complementary food based on wheat and soyabean was demonstrated to be significantly enhanced after dephytinisation (Egli, 2001).

Although the inhibitory effect of phytic acid has not previously been reported in infants, studies in young animals (rat pups and infant rhesus monkeys) demonstrated increased Zn absorption from low-phytic-acid soya formula (Lönnerdal *et al.* 1988, 1999). The phytic acid contents were 410 v. 44 mg/l (Lönnerdal *et al.* 1988) and 300 mg v. 60 mg/l (Lönnerdal *et al.* 1999) before and after dephytinisation respectively. The lack of effect of partial reduction of phytic acid in soya formula on Zn absorption in infants reported by Ziegler *et al.* (1990) was probably due to the relatively high content of phytic acid present in both products: 123 and 292 mg phytic acid/l respectively.

Our previous studies in infants demonstrated that dephytinisation of soyabean protein isolate could be a useful approach to improve Fe bioavailability from soya formula as Fe absorption, measured by a stable isotope technique based on erythrocyte incorporation of Fe, increased significantly after degradation of 100 % or 83 % phytic acid (Davidsson *et al.* 1994). The lack of effect of dephytinisation on Fe absorption in the present study was therefore somewhat surprising. However, it is important to stress that the native phytic acid content in the soya formulas was about 30 % greater in the study of Davidsson *et al.* (1994) than in the present study. These results indicate that the effect of dephytinisation is more pronounced when applied to soyabean protein isolate with higher native content of phytic acid. Furthermore, ascorbic acid is a potent enhancer of Fe absorption that can overcome the inhibiting effect of phytic acid when present in high enough quantities. For example, our previous studies in infants demonstrated that Fe bioavailability could be enhanced to the same extent by dephytinisation or by increasing the ascorbic acid:Fe molar ratio from 2:1 to 4:2 (Davidsson *et al.* 1994). In the present study, ascorbic acid was obviously effective in counteracting the inhibitory effect of phytic acid on Fe absorption in the regular soya formula. These results are thus in agreement with a previous study where no additional effect of dephytinisation of an infant cereal with low native phytic acid content (0.08 %) and added ascorbic acid (molar ratio 2:1 relative to Fe) was observed in infants (Davidsson *et al.* 1997). In the present study we evaluated Fe absorption by a stable isotope technique based on erythrocyte incorporation of ^{57}Fe and ^{58}Fe as well as by faecal excretion of the isotopes. No statistically significant difference in Fe absorption was observed between the two soya formulas with either stable isotope technique. However, our observation that fractional Fe absorption differed based on erythrocyte incorporation as compared with results based on faecal excretion are in agreement with the previous study by Fomon *et al.* (2000) and highlight some of the

methodological limitations of using erythrocyte incorporation as a proxy for Fe absorption in infants.

Very limited information is available on Cu absorption in infants and no results on the effect of phytic acid on Cu absorption during early life have been reported previously. Our present results are in agreement with earlier studies in adult human subjects (Turnlund *et al.* 1985; Egli, 2001) and in rat pups and infant rhesus monkeys (Lönnerdal *et al.* 1999), demonstrating no inhibiting effect of phytic acid on Cu absorption. Furthermore, only limited information is available on Ca absorption from soyabean products in human subjects and, to our knowledge, the effect of complete dephytinisation of soya formulas on Ca absorption has not been evaluated previously in infants. However, Heaney *et al.* (1991) reported significantly greater Ca absorption from soyabeans with low phytic acid content as compared with soyabeans with higher phytic acid content in adult women. Ca absorption measured by a stable isotope technique was relatively high in the present study: mean values were about 65 % for both soya formulas, and were similar to recent results on Ca absorption from a cows'-based infant formula containing lactose (Abrams *et al.* 2002). The study by Abrams *et al.* (2002) reported Ca absorption, measured by a stable isotope technique, to be 66 (SD 12) % in eighteen infants. The results from the present study thus indicate that modern soya infant formulas provide similar amounts of absorbed Ca as cows'-based formula and that Ca absorption is not inhibited from soya formula based on soyabean protein isolate with low native content of phytic acid.

In conclusion, the aim of the present study was to investigate the effect of dephytinisation of soyabean protein isolate on mineral and trace element absorption in healthy infants fed soya formula as part of the ongoing efforts to improve the nutritional quality of commercially produced infant foods. However, the nutritional benefit of dephytinisation was marginal in the present study. The results clearly indicate that the use of soyabean protein isolate with low native phytic acid content should be promoted for production of infant formulas and adequate addition of ascorbic acid to enhance Fe absorption should be ensured in the products. However, dephytinisation as a strategy to improve mineral and trace element absorption from foods with high phytic acid content, for example complementary foods based on cereals and legumes consumed by infants and young children in developing countries, should be evaluated for long-term effects on childhood nutrition and health.

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